Figure S1

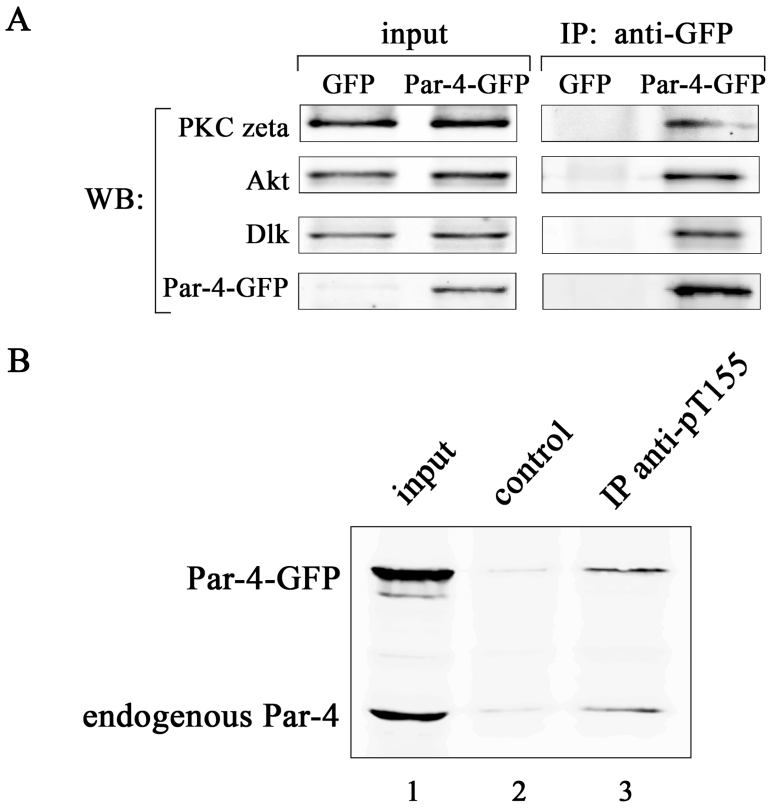

Figure S1. Control experiments for the GFP-tagged Par-4 wt construct. (A) Binding of Par-4 to its interaction partners Dlk, Akt and PKC zeta is not affected by the GFP tag. REF52.2 cells were transfected with GFP or Par-4-GFP wt. Lysates were subjected to immunoprecipitation with a polyclonal anti-GFP antibody (Invitrogen). Immunoprecipitates were washed three times with buffer and analyzed by Western blot analysis using a rabbit polyclonal Dlk antibody at 1:1000 dilution (Cell Signaling), a rabbit polyclonal anti-PKC zeta antibody at 1:500 dilution (Invitrogen), a rabbit polyclonal anti-Akt antibody at 1:500 dilution (Cell Signaling) and the the mouse monoclonal anti-Par-4 antibody at 1:500 dilution (Santa Cruz). The input (left panel) represents 20 µg of whole cell lysate per lane. (B) Phosphorylation of Par-4 is not affected by the GFP-tag. REF52.2 cells were transfected with Par-4-GFP. Cells were serum-starved for 16 h with subsequent stimulation with 10 µM lysophosphatidic acid (LPA) for 15 minutes to induce Par-4 phosphorylation. 24 h post-transfection, cells lysates were subjected to immunoprecipitation with the phospho-specific antibody Par-4(P)T155 (lane 3, Pineda Antibody Service, Berlin, Germany) or pre-immune serum (control, lane 2). Immunoprecipitates were analyzed by Western blot analysis with the mouse monoclonal anti-Par-4 antibody (1:500, Santa Cruz). The input (lane 1) represents 20 µg of whole cell lysate. Please note that both endogenous and transfected Par-4 are specifically immunoprecipitated by the phospho-specific antibody Par-4(P)T155 suggesting a comparable level of phosphorylation after LPA stimulation.

Figure S2

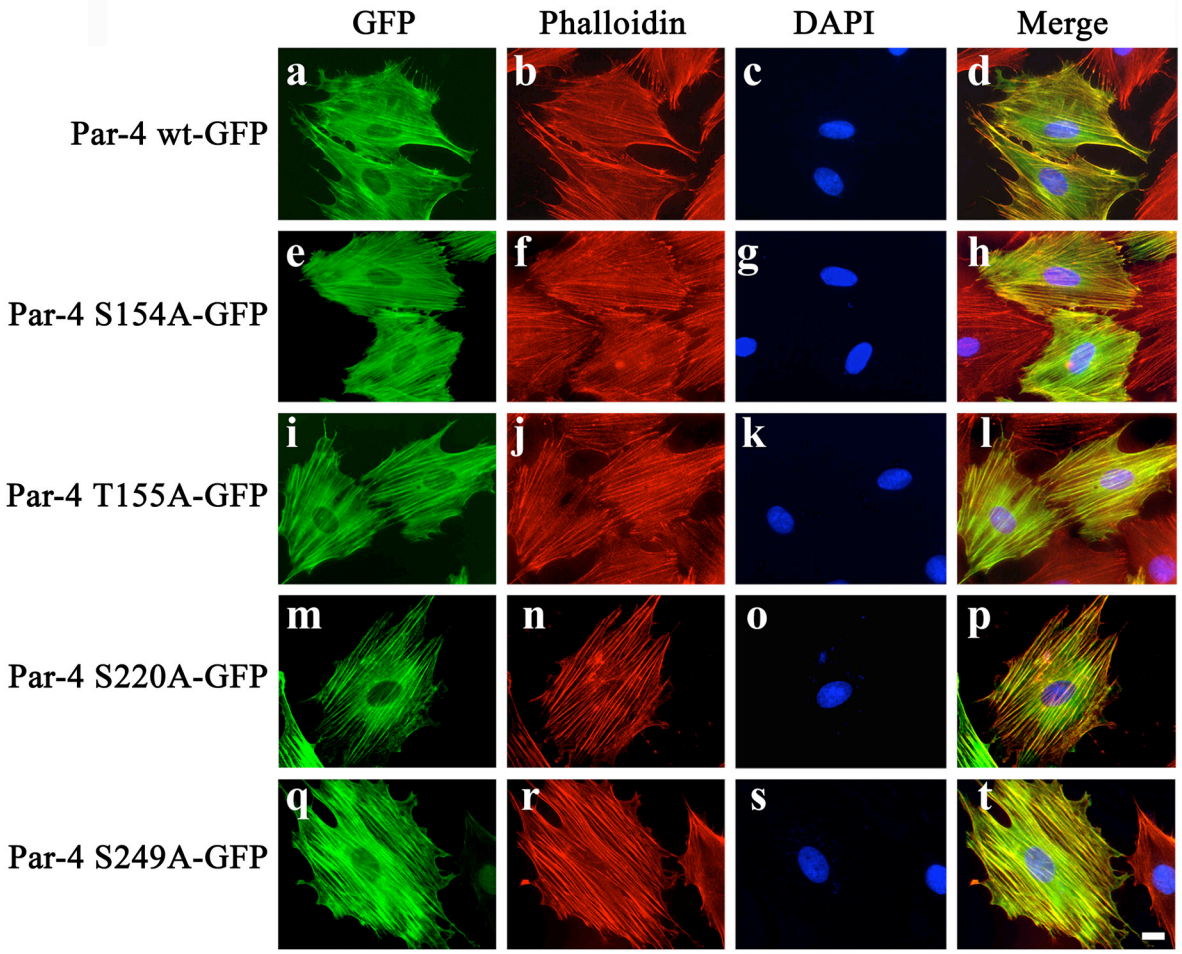

Figure S2. Subcellular localization of Par-4 wt and different Par-4 phospho-mutants in rat fibroblasts. REF52.2 cells were transiently transfected with C-terminally GFP-tagged constructs of Par-4 wt (a-d), Par-4 phospho-mutant S154A (e-h), T155A (i-l), S220A (m-p) and S249A (q-t). 24 h after transfection, the cells were fixed with formaldehyde, stained with TRITC-labeled phalloidin (b, f, j, n and r) to determine the subcellular localization of each Par-4 phospho-mutant and with DAPI (d, h, l, p and t). Note, that all Par-4 phospho-mutants showed clear association with actin filaments. Scale bar, 10 µm.

Figure S3

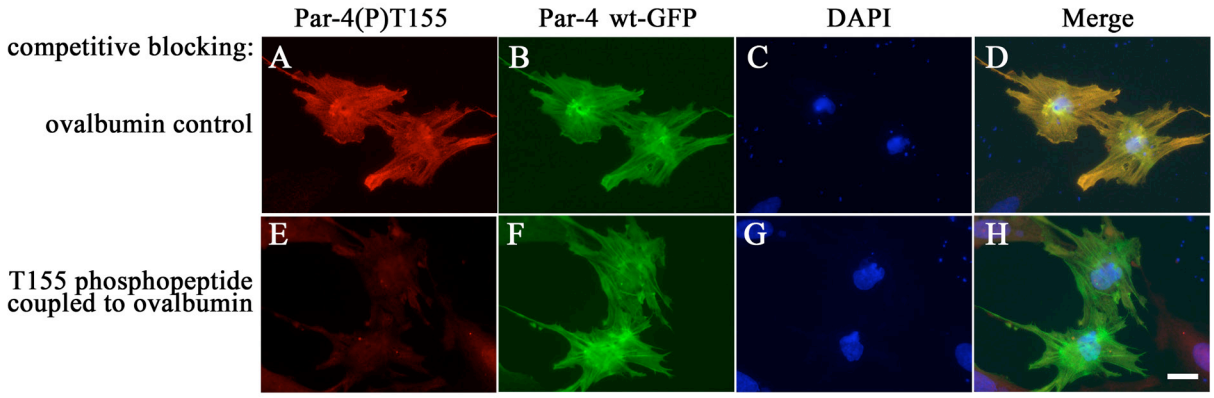

Figure S3. Blocking experiment to test antibody specificity. The phospho-specific antibody Par-4(P)155 diluted 1:2000 in PBS and preincubated for 1 h with the phospho-peptide KRRSpTGVVN coupled to ovalbumin at a ovalbumin concentration of 2.5 µg/ml. As control, the antibody was preincubated with ovalbumin alone. The preincubated antibodies were then used to stain REF52.2 cells co-transfected with Par-4-GFP and FLAG-Dlk at a cDNA ratio of 3:1. Cells were stained with DAPI to visualize nuclei. Please note that the specific signal produced by the anti-Par-4(P)T155 antibody is completely abolished by preincubation of the antibody with the ovalbumin-coupled phosphopeptide (E), but not in the control experiment with ovalbumin alone (A). Scale bar, 20 µm.